

Comparative Spermatozoal Ultrastructure and its Taxonomic and Phylogenetic Significance in The Bivalve Order Veneroida

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ABSTRACT

A comparative study of sperm ultrastructure in the Veneroida, an ecologically and economically important order of bivalve molluscs, has revealed significant differences between taxa. Most veneroid spermatozoa are of the aquasperm type (general features: conical acrosome, nucleus usually short, midpiece consisting of a ring of round mitochondria surrounding the centrioles, single simple flagellum). Five principle sperm morphologies can be recognized in the Veneroida, these correlating to varying degrees of precision with groups of superfamilies. Group A (Lucinoidea, Cardioidea (including Tridacnidae), Veneroidea, Mactroidea, Chamoidea, Solenoidea, Tellinoidea (one species of Donacidae)): basal ring of acrosome without visible substructure and not developed longitudinally; nucleus curved if rod-shaped. Group B (Galeommatoidea (*Mysella*, *Scintilla*, *Divariscintilla*)): sperm similar to Group A and especially C, but with strongly tilted acrosomal complex and basal ring developed transversely. Group C (Tellinoidea (Donacidae, Tellinidae), Arcticoidea, Dreissenoida, Galeommatoidea (*Lasaea*)): basal ring developed longitudinally, sometimes showing substructure; overlap between mitochondria and nucleus only in Tellinidae. Group D (Tellinoidea (Scrobiculariidae), Corbiculoidea): acrosome and nucleus elongate; pronounced overlap of mitochondria with nucleus. Group E (Carditoidea + Crassatelloidea assemblage): acrosome and nucleus elongate; midpiece exhibiting usually 8 mitochondria; proximal centriole modified into well developed rootlet. The widespread occurrence of the Group A spermatozoon within the Veneroida including the basal superfamily Lucinoidea, suggests that this sperm type was typical of early veneroids. In contrast, the sperm morphologies encountered in Groups D and E are unknown elsewhere within the Bivalvia, and undoubtedly represent modifications from a less complex sperm type (e.g. Group A sperm).

RÉSUMÉ

Ultrastructure comparée des spermatozoïdes et sa signification taxonomique et phylogénétique dans l'ordre des Veneroida (Bivalves)

L'étude comparée de l'ultrastructure des spermatozoïdes chez les Veneroida, un groupe de Mollusques important du point de vue écologique et économique, a montré des différences significatives entre les taxons. La plupart des spermatozoïdes des Veneroida sont du type aquaspermatozoïde, dont les caractéristiques générales sont un acrosome conique, un noyau généralement court, une pièce intermédiaire consistant en un anneau de mitochondries rondes entourant les centrioles et un flagelle simple et unique. Cinq morphologies principales de spermatozoïdes peuvent être reconnues chez les Veneroida, et peuvent être corrélées avec des degrés variés de précision avec les groupes ou les superfamilles. Groupe A (Lucinoidea, Cardioidea (y compris les Tridacnidae), Veneroidea, Mactroidea, Chamoidea, Solenoidea, Tellinoidea (une espèce de Donacidae)): anneau basal de l'acrosome sans substructure visible et non développé longitudinalement; noyau courbe si en

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forme de baguette. Groupe B (Galeommatoidea (*Mysella*, *Scintilla*, *Divariscintilla*)): spermatozoïde similaire au groupe A et spécialement au groupe C, mais avec un complexe acrosomien fortement incliné et un anneau basal développé transversalement. Groupe C (Tellinoidea (Donacidae, Tellinidae), Arcticoidea, Dreissenoida, Galeommatoidea (*Lasaea*)): anneau basal développé longitudinalement, montrant parfois une substructure; chevauchement entre les mitochondries et le noyau seulement chez les Tellinidae. Groupe D (Tellinoidea (Scrobiculariidae), Corbiculoidea): acrosome et noyau allongés; chevauchement prononcé des mitochondries et du noyau. Groupe E (Carditoidea + assemblage des Crassatelloidea): acrosome et noyau allongés; pièce intermédiaire montrant généralement huit mitochondries; centriole proximal modifié en une racine bien développée. La présence très répandue du spermatozoïde du groupe A chez les Veneroida, y compris dans la superfamille primitive Lucinoidea, suggère que ce type de spermatozoïde était typique des premiers Veneroida. Au contraire, les morphologies de spermatozoïdes rencontrées dans les groupes D et F sont inconnues dans d'autres groupes des Bivalvia, et représentent de manière certaine des modifications à partir d'un type de spermatozoïde moins complexe (par exemple le spermatozoïde du groupe A).

The Veneroida constitute one of the most important extant orders of bivalve molluscs. Included within the group are several marine families of economic and ecological significance such as the cockles and giant clams (Cardiidae, Tridacnidae), venus shells (Veneridae and allies), tellins (Tellinidae and allies) and the estuarine/ freshwater family Corbiculidae. Because of this, studies of veneroid reproductive biology are of considerable importance in understanding the success of the order. In addition, comparative work on sperm fine structure in this and other molluscan groups continues to generate characters of taxonomic and phylogenetic significance, most notably in the internally fertilizing Gastropoda which have complex, often polymorphic, spermatozoa [12, 13, 17, 25, 26]. Although sperm morphology has been examined for several bivalve species, it is only in recent years that a comparative approach has been applied within this class at the ultrastructural level. In 1971 GHARAGOZLOU-VAN GINNEKEN & POCHON-MASSON [10] were the first authors to demonstrate significant differences between species and genera within the Veneridae (Veneroidea, Veneroida, see Table 1). Similar studies have been carried out by Hodgson and co-workers [19-21] on various Veneroida (congeneric species of Solenidae and Donacidae, see Table 1) and Mytiloidea. Such information is not only useful in verifying the validity of species, but also offers an opportunity to test their relationships to other congeners - the latter exercise being dependent on the availability of other sperm data. On a broader scale, sperm ultrastructural studies by POPHAM [41] and HEALY [14, 17] have also been directed towards investigating the taxonomic and evolutionary relationships between bivalve subclasses and orders.

Above the species and genus levels, sperm ultrastructure appears to be a promising source of taxonomic and phylogenetic information in the Veneroida based on my own observations (presented here, see Table 1) as well as data from published accounts (Table 2). The present study examines comparative sperm morphology among veneroid bivalves and concludes with a discussion of the possible taxonomic and phylogenetic implications of all available data for the group. Although some authors exclude the Lucinoidea from the Veneroida (placed in a separate order by MORRIS [33], in a separate subclass by POJETA [40]), I have adopted the more traditional approach [1, 5, 32, 49] and retained it within the order.

MATERIAL AND METHODS

The present survey of veneroid sperm ultrastructure is based on the author's observations (using species collected from the Queensland coast: see Table 1) combined with information already available in the literature (see Table 2). For most species investigated herein, fixation of testicular tissue was carried out using ice cold (0-4°) glutaraldehyde (3.5% in 0.1 M phosphate buffer containing 10% w/v sucrose) followed by placing tissue pieces into 1% osmium tetroxide (at 0-4°C, in 0.1 M sucrose adjusted phosphate buffer), followed by three rinses in phosphate buffer (at 0-4°C, buffer sucrose adjusted), ethanol dehydration and embedding in Spurr's epoxy resin. For *Codakia punctata* (Lucinoidea) and *Tellina rostrata* (Tellinoidea) sea water formalin testis tissues only were processed for TEM. Semithin and ultrathin sections were cut using an LKB UM IV Ultratome stained according to the procedure of DADDOW [7] and examined using an Hitachi H-300 transmission electron microscope.

TABLE 1. —Veneroid taxa examined in the present study and dimensions of acrosome, nucleus and midpiece (dimensions averaged, $n = 5$). ND = not determined. Dimensions expressed in μm . L = maximum length; D = maximum diameter. Maximum diameter measurements for acrosomal vesicle and nucleus taken through base of these sperm components.

Taxa	Acrosomal Vesicle (LxD)	Nucleus (LxD)	Midpiece (LxD)
Superfamily Lucinoidea			
Family Lucinidae			
<i>Codakia punctata</i>	0.50 x 0.95	3.68 x 1.20	1.00 x 1.40
Superfamily Cardioidea			
Family Cardiidae			
<i>Fragum hemicardium</i>	0.25 x 0.60	2.16 x 1.55	0.68 x 1.75
<i>Fragum unedo</i>	0.27 x 0.67	2.20 x 1.35	0.53 x 1.70
<i>Acrosterigma reeveanum</i>	0.34 x 0.46	ND	ND
Family Tridacnidae			
<i>Tridacna maxima</i>	0.50 x 0.70	ND	ND
Superfamily Mactroidea			
Family Mactridae			
<i>Spisula trigonella</i>	0.44 x 0.38	1.25 x 2.00	1.00 x 2.25
Superfamily Veneroidea			
Family Veneridae			
<i>Placamen calophyllum</i>	0.73 x 0.46	3.06 x 1.00	0.53 x 0.70
<i>Circe cf plicatina</i>	0.64 x 0.74	3.0 x 1.35	0.64 x 1.70
<i>Paphia</i> sp.	0.67 x 0.56	1.8 x 1.52	0.60 x 1.60
Family Glauconomidae			
<i>Glauconome</i> sp.	1.20 x 0.37	4.50 x 0.75	1.66 x 0.66
Superfamily Tellinoidea			
Family Donacidae			
<i>Donax deltoides</i>	1.18 x 0.88	0.67 x 0.75	0.60 x 1.75
Family Tellinidae			
<i>Tellina rostrata</i>	0.55 x 0.45	0.00 x 0.70	1.56 x 0.82
Superfamily Arcticoidea			
Family Trapeziidae			
<i>Trapezium sublaevigatum</i>	0.45 x 0.56	2.00 x 1.20	0.46 x 1.36

TABLE 2. — Veneroid taxa previously investigated for sperm ultrastructure

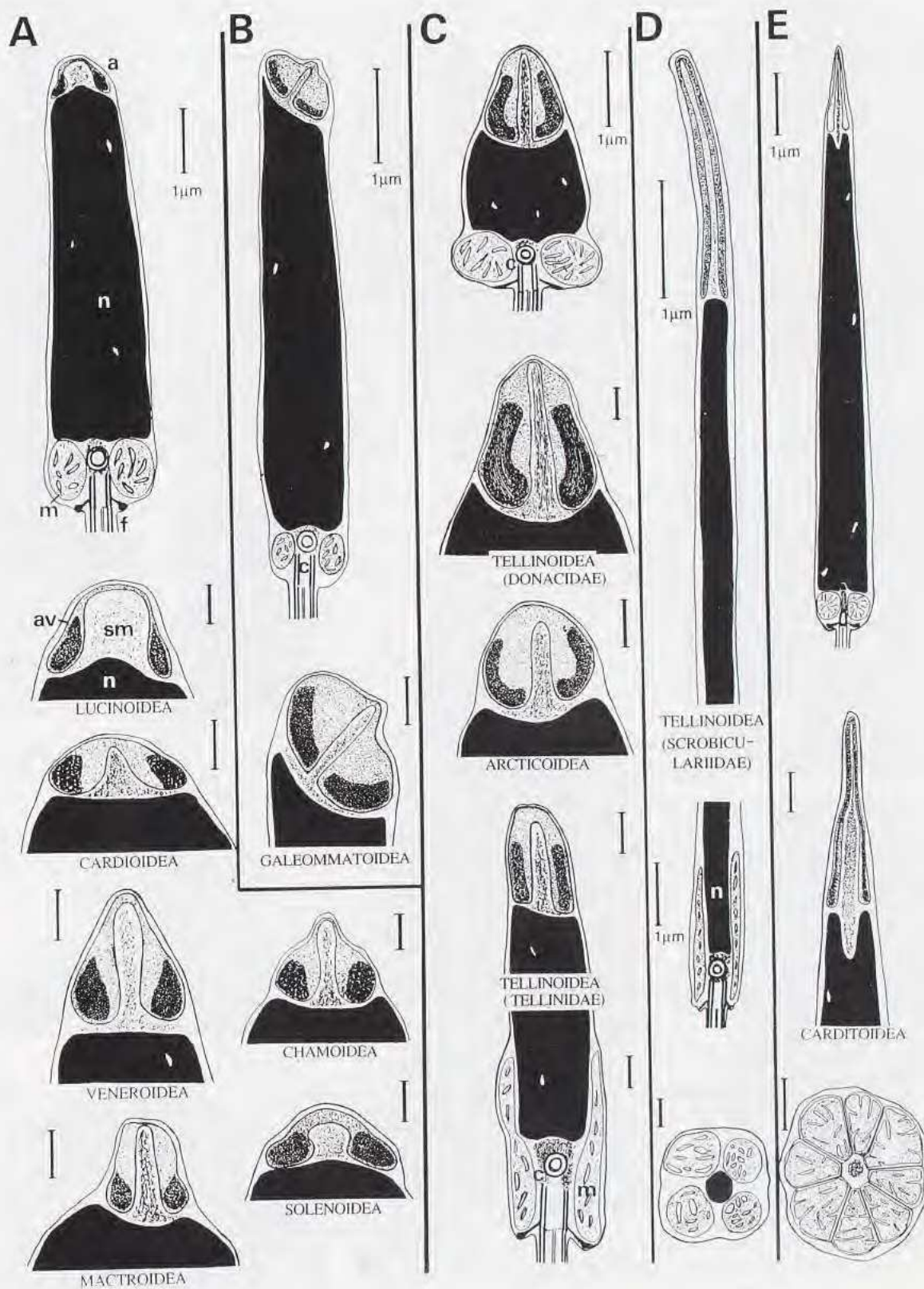
Superfamily Cardioidea		<i>Donax serra</i>	[20]
Family Cardiidae		<i>Donax trunculus</i>	[47]
<i>Fulvia tenuicostata</i>	[41]	Family Scrobiculariidae	
<i>Cardium edule</i>	[46]	<i>Scrobicularia plana</i>	[48]
Superfamily Mactroidea		Superfamily Dreissenoidea	
Family Mactridae		Family Dreissenidae	
<i>Spisula solidissima</i>	[22, 29]	<i>Dreissena polymorpha</i>	[9]
Superfamily Veneroidea		Superfamily Galeommatoidea	
Family Veneridae		Family Galeommatidae	
<i>Venerupis aurea</i>	[10]	<i>Lasaea subviridis</i>	[35, 38]
<i>Venerupis corrugata</i>	[10]	<i>Mysella tumida</i>	[35, 36]
<i>Ruditapes decussatus</i>	[10, 39]	<i>Pseudopythina rugifera</i>	[37]
<i>Tapes rhomboides</i>	[10]	<i>Divariscintilla yoyo</i>	[8]
<i>Callista chione</i>	[34]	<i>Divariscintilla troglodytes</i>	[8]
<i>Tivela polita</i>	[43]	<i>Scintilla</i> sp.	[8]
Superfamily Solenoidea		Superfamily Corbiculoidea [13]	
Family Solenidae		Family Corbiculidae (<i>Corbicula</i>)	
<i>Solen cylindraceus</i>	[21]	<i>Corbicula sandai</i>	[11]
<i>Solen capensis</i>	[21]	<i>Corbicula fluminea</i>	[28]
<i>Ensis ensis</i>	[4]		
Superfamily Chamoidea		Superfamily Carditoidea	
Family Chamidae		Family Carditidae	
<i>Chama macerophylla</i>	[22]	<i>Cardita muricata</i>	[18]
Superfamily Tellinoidea		Superfamily Crassatelloidea	
Family Donacidae		Family Crassatellidae	
<i>Donax madagascariensis</i>	[20]	<i>Eucrassatella cuningii</i>	[18]
<i>Donax sordidus</i>	[20]	<i>Eucrassatella kingicola</i>	[18]
		<i>Talabrica aurora</i>	[18]

RESULTS AND DISCUSSION

Aquasperm features of veneroid spermatozoa

Most veneroid spermatozoa, like those of the majority of other bivalve taxa, could be classed as unmodified or relatively unmodified aquasperm, the principal features of which are as follows: (1) a well developed, conical acrosomal vesicle; (2) a short or relatively short nucleus (with electron-lucent lacunae); (3) a short midpiece (containing round mitochondria, usually four or five in number, surrounding a pair of orthogonally arranged, triplet substructure centrioles); (4) a radial array of satellite fibres anchoring the distal centriole (basal body) to the plasma membrane; (5) a simple flagellum (9+2 axoneme sheathed only by the plasma membrane). Although most differences in sperm morphology between veneroid taxa involve acrosomal and/or nuclear

FIG. 1. — A-E: The five major sperm morphologies occurring within the Veneroida. a, acrosomal complex; av, acrosomal vesicle; c, centrioles (proximal and distal); f, flagellum; m, mitochondria (of midpiece); n, nucleus; sm, subacrosomal material. Scale bars = 0.25 µm, except where indicated. Sources of data: present study except Chamoidea [2]; Solenoidea [4]; Galeommatoidea [5, 6]; Tellinoidea - Scrobiculariidae [9].



features, some useful variations in midpiece architecture are also apparent such as differing mitochondrial number and presence/absence of an unmodified proximal centriole. The five main sperm morphologies occurring in the Veneroidea, and their chief characteristics, are as presented below (taxa studied here indicated by *, other sources referenced by number).

Group A: Lucinoidea [*], *Cardioidea* [*, 46], *Veneroidea* [*, 10, 20, 34, 39, 43], *Mactroidea* [*, 22, 29], *Tellinoidea* (*Donacidae* - *Donax trunculus* only, [47]), *Chamoidea* [22], *Solenoidea* [4, 21] (Figs 1A, 2A-E)

General features: (1) acrosomal vesicle short, conical, deeply invaginated, with thick, highly electron-dense basal ring (longitudinal profile of ring round to pyriform); subacrosomal material either diffuse or with axial rod differentiated; (2) nucleus short or rod-shaped (usually curved if rod-shaped) depending on family or genus, typically with no or only a poorly developed apical depression (apex often convex); (3) midpiece with two unmodified (triplet substructure) centrioles surrounded by four or most commonly five rounded mitochondria; (4) satellite fibre complex anchoring distal centriole to plasma membrane; (5) single flagellum with 9+2 axoneme.

The widespread occurrence of this sperm morphology within the Veneroidea, including the Lucinoidea (considered by many authors as the oldest extant veneroid superfamily), suggest that it was probably characteristic of the earliest members of the order. Variation in the shape and dimensions of the acrosomal vesicle and nucleus is considerable in the Veneroidea.

Group B: Galeommatoidea (*Lasaea*, *Scintilla*, *Divariscintilla*) [8, 35]. (Fig. 1B)

Features as for Groups A and especially C, but differing in having the acrosomal complex arranged at a considerable angle to the longitudinal axis of the spermatozoon. Basal ring crescentic (as in Group C) but developed transversely.

This type of sperm morphology was probably derived from the more widespread Group C type, through re-alignment of the acrosomal vesicle (and its crescentic basal ring). A detailed study of *Scintilla* and *Divariscintilla* [8] shows that the tilted positioning of the acrosomal vesicle seen in mature spermatozoa takes place in the final phase of spermiogenesis.

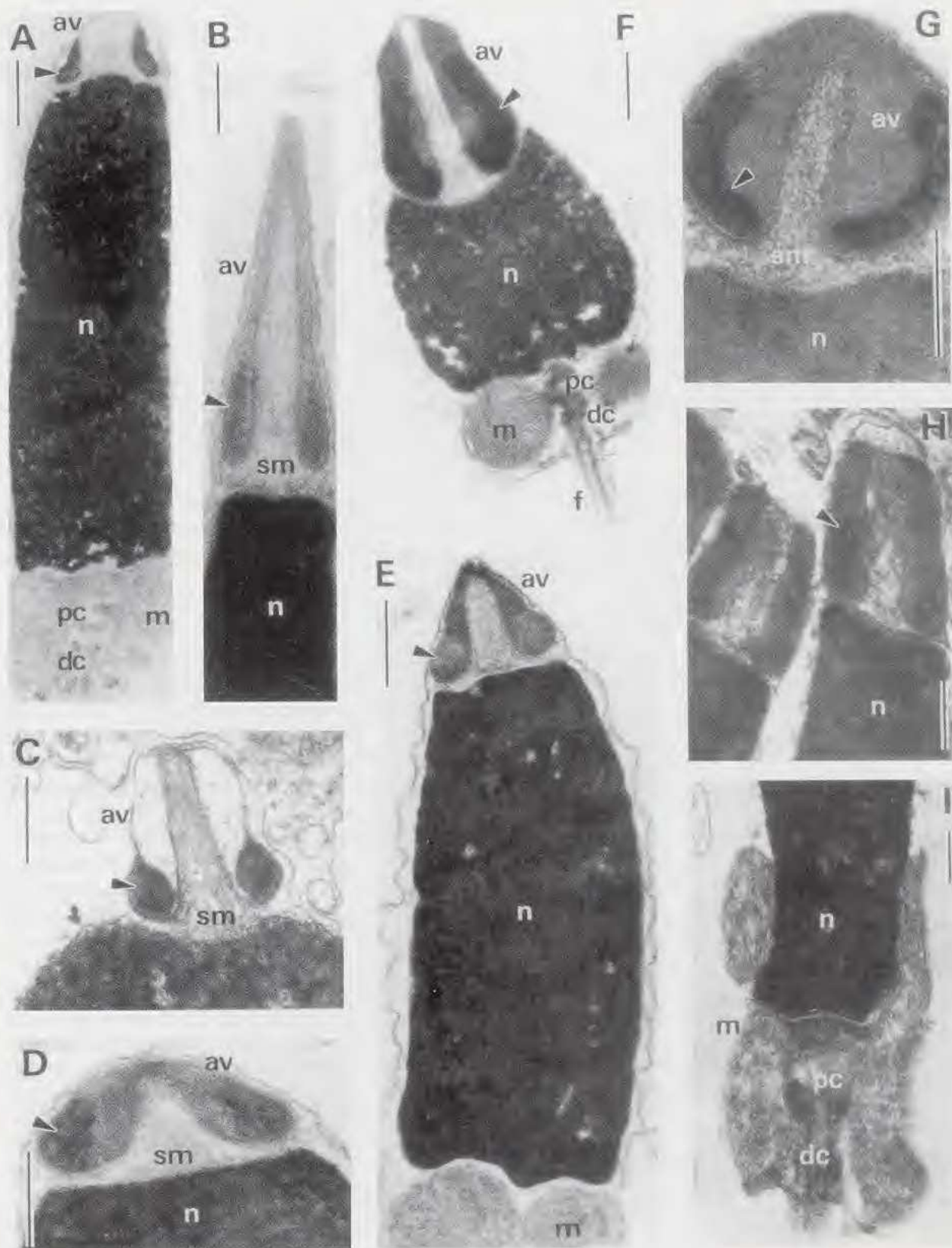
Group C Tellinoidea (*Donacidae* [20,*], *Tellinidae* [*]), *Arcticoidea* [*], *Dreissenoidae* [9], *Galeommatoidea* (*Mysella* [35]). (Figs 1C, 2F-I)

Features resembling those of Groups A and especially B. Group C acrosomes differs from those of Group B in not being tilted and in having the basal ring developed longitudinally rather than transversely. Substructure sometimes visible within the acrosomal vesicle contents. Marked overlap of midpiece and nucleus in *Tellina* (*Tellinidae*, *Tellinoidea*) similar to Group D.

Group D: Tellinoidea (*Scrobiculariidae* [48]), *Corbiculoidea* [11, 28]. (Fig. 1D)

Acrosomal vesicle slender, almost totally invaginated with basal ring not clearly defined. Subacrosomal material diffuse, without axial rod. Nucleus elongate and slender. Midpiece

FIG. 2. — A-E: Electron micrographs illustrating major variations between Veneroidea examined. Group A. A: Sperm head and midpiece of *Codakia punctata* (Lucinidae, Lucinoidea). B: Acrosome of *Glaucanome* sp. (Glauconomidae, Veneroidea). Arrowhead in this and other figures indicates basal ring component of acrosomal vesicle contents. C: Acrosome of *Spisula trigonella* (Mactridae, Mactroidea). D: Acrosome of *Fragum unedo* (Cardiidae, Cardioidea). E: Sperm head and midpiece of *Circe cf plicatina* (Veneridae, Veneroidea). Note curved nucleus. F: Sperm head, midpiece and proximal portion of flagellum of *Donax deltoides* (Donacidae, Tellinoidea). G: Acrosome of *Trapezium sublaevigatum* (Trapeziidae, Arcticoidea). H: Acrosome of *Tellina rostrata* (Tellinidae, Tellinoidea). I: Nuclear base and midpiece of *T. rostrata*. Note extensive overlap of mitochondria and nucleus. av, acrosomal vesicle; dc, distal centriole; f, flagellum; m, mitochondria (of midpiece); n, nucleus; pc, proximal centriole; sm, subacrosomal material. Scale bars: A, E, F = 0.5 µm; B-D, G-I = 0.25 µm.



mitochondria oblong and overlapping significantly with base of nucleus (similar to *Tellina* from Group C).

This represents one of the most modified sperm morphologies encountered in the Veneroida. The type is of special interest because microtubules surround the spermatid nucleus during condensation (at least in *Scrobicularia*; details unavailable for *Corbicula*). Spermatid perinuclear microtubules occur in no other bivalves, but are widely reported in gastropods (introsperm-producing taxa [15, 25, 26]), cephalopods [16] and polyplacophorans [3]. The presence of this sperm type in the Tellinoidea and Corbiculoidea raises important questions concerning the relationship between these superfamilies (see Systematic considerations below). A report (based on SEM and light microscopy) that spermatozoa of *Corbicula fluminea* are both dimorphic (head region either slender or wide) and biflagellate [28], requires confirmation using transmission electron microscopy.

Group E: Carditoidea (Carditidae, Crassatellidae) [18]. (Fig. 1E)

Acrosomal vesicle elongate conical, almost totally invaginated, with the presumed homologue of the basal ring evident as a dense inner layer. Subacrosomal material differentiated into axial rod. Nucleus rod-shaped with short but distinct apical depression (partly accommodating subacrosomal material). Midpiece characterized by 8 (rarely 7 or 9) tightly adpressed mitochondria surrounding a dense centriolar rod (a metamorphosed proximal centriole) and distal centriole.

This is a highly distinctive sperm type, and its presence in the Carditidae and Crassatellidae has wide taxonomic and phylogenetic implications within the Veneroida (see below). The transformation of the proximal centriole into a rod connecting distal centriole (basal body) to nuclear fossa is unique among molluscan aquasperm.

Phylogenetic and systematic considerations

According to ALLEN [1] the success of veneroid bivalves has pivoted on their exploitation of soft sediments, largely as a consequence of their possessing and developing siphons (in combination, it should be stressed, with an ability to burrow effectively). Although most adaptive radiation within the Veneroida took place within the Mesozoic, some superfamilies such as the Lucinoidea and Crassatelloidea extend back to the lower Palaeozoic [1, 6, 31, 32] while several important living families and genera date only from the late Cretaceous or Tertiary [31]. Veneroid origins are unclear, and it remains uncertain as to whether the group is truly monophyletic or not.

In the present account, five principal sperm morphologies are recognized within the Veneroida based on shared ultrastructural features (observed through TEM). Light microscopic work of KARPEVICH [23] indicates that helical sperm nuclei also occur in several species of Cardiidae and at least one species of Tellinidae. Present knowledge of comparative sperm ultrastructure in the Veneroida, despite the absence of data for a number of families and genera, provides new information relevant to discussions of relationships within the order.

Group A. Despite anatomical specializations in some living representatives, the Lucinoidea constitute one of the oldest living heterodont superfamilies, being definitely recorded from the Silurian [1, 5] but possibly extending back to the Ordovician if a close relationship with the fossil genus *Babinka* is accepted [30, 31, 40]. The presence of Group A sperm morphology in this superfamily, and its widespread occurrence within the Veneroida, suggest that the earliest members of the order also possessed Group A type spermatozoa. Comparative spermatology in Group A superfamilies, especially in relation to acrosomal and nuclear features, appears to have considerable taxonomic potential at the species and generic levels (e.g. in the Veneroidea [10]; Cardioidea [*]; Solenoidea [21]). The disputed superfamily placement of the Hemidonacidae [45], either among the Cardioidea or the Tellinoidea, could probably be settled by examination of sperm ultrastructural features, although it should be added that within at least one tellinoidean family

(Donacidae) the acrosome may be of Group A type (*Donax trunculus* only - [47]) or Group C (all four other investigated species of *Donax*).

Group B. The Galeommatoidea (= Leptonacea) are small, often hermaphroditic veneroids which fertilize eggs within the mantle cavity (where young are brooded) and employ a range of methods to transfer sperm from individual to individual (spermatophores, sperm morulae, usage of dwarf males) [35-38]. In addition, the only confirmed cases of sperm dimorphism within the Bivalvia involve galeommatoidean species [35].

The classification of galeommatoidean bivalves (= Leptonacea) is far from being fully resolved [2, 8], and this seems to be reflected in marked acrosomal differences between examined genera. For example, in *Lasaea*, *Scintilla* and *Divariscintilla* (Group B), the acrosomal complex is apically compressed and tilted at a considerable angle. In addition the basal ring is developed transversely (Fig. 1B). By contrast, in *Mysella* (placed in Group C), the acrosomal vesicle tapers apically and is not tilted. The basal ring is developed longitudinally and accompanied by a lamellate apical density [35]. Unfortunately the only available TEM micrograph of *Pseudopythina* spermatozoa [37] is not detailed enough to determine the substructure of the acrosome. Although galeommatoideans probably arose through neoteny [1], the actual source (? or sources) of these bivalves has not been identified with any certainty. Available sperm data suggest that all galeommatoideans could have arisen from the Tellinoidea or the Arcticoidea, or possibly from both if the superfamily proves not to be monophyletic.

Group C. Spermatozoa of Group C could have been easily derived from those of Group A through lengthening and an increase in complexity of the basal ring component of the acrosomal vesicle. Within the Tellinoidea, spermatozoa of the Donacidae are typical of Group C, while those of *Tellina rostrata* (Tellinidae) appear to bridge the gap between Groups C and D by having significant mitochondrial overlap with the nucleus. It is unfortunate that so few tellinoidean families have been examined for sperm ultrastructure (e.g. no available data for the Solecurtidae, Semelidae, Psammobiidae). Such information would be of considerable value not only in evaluating relationships within the Tellinoidea, but also in exploring possible connections with the Arcticoidea, Galeommatoidea and freshwater Dreissenioidea. At the species level, comparative sperm morphology within the Donacidae may prove to be of considerable taxonomic use. Spermatozoa of the Australian species *Donax deltoides* are structurally similar to, although not identical with, those of the South African species *D. sordidus* and *D. madagascariensis* but differ markedly in acrosomal shape from another South African species *D. serra* (compare Fig. 1C, 2F with results by HODGSON *et al.* [20]). Spermatozoa of all four of these species of *Donax* differ from those of *D. trunculus*, which has a more elongate nucleus and an acrosome essentially of the Group A type [47].

Group D. Particularly interesting is the similarity between spermatozoa of the tellinoidean *Scrobicularia plana* (the only investigated member of the Scrobiculariidae) (Fig. 1D) and the freshwater Corbiculoidea. Is it possible that the Corbiculoidea have been derived from the Tellinoidea, or are the observed sperm similarities between *Scrobicularia* and *Corbicula* merely the result of convergence (that is, a similar fertilization biology)? In the absence of comprehensive data for a range of tellinoidean and corbiculoidean species this must remain an intriguing but presently insoluble problem. BOSS [2] considered that the Scrobiculariidae were perhaps unnecessarily split from the large tellinoidean family Semelidae. Further research on the spermatozoa of semelids and scrobiculariids (Tellinoidea) will undoubtedly throw further light onto this issue.

Group E. A close relationship between the Carditidae and Crassatellidae is clearly indicated by sperm morphology providing strong support for YONGE's suggestion [50, 51] that the Carditoidea and Crassatelloidea should be united into a single superfamily (for further discussion see HEALY [18]). Although the relationship of Group E (Carditidae + Crassatellidae) to other Veneroida is extremely uncertain, the preponderance of apomorphic sperm characters in this

Group effectively excludes it as a source of other veneroid taxa (e.g. origin of Veneroidea, Tellinoidea, Chamoidea plus Myoida from the "Carditida" suggested by SCARLATO & STAROBOGATOV [44]; origin of Cardioidea from carditids or crassatellids suggested by KEEN [24], origin of Tridacnidae from Carditoidea suggested by ALLEN [1]).

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